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Occurrence and Frequency of Coronavirus Infections in Humans as Determined by Enzyme-Linked Immunosorbent Assay

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The occurrence of human coronavirus (HCV) infections was analyzed by using sequential sera taken between 1976 and 1981 from adults working in the London area. Antibody rises to HCV 229E and HCV OC43 group viruses were measured in serum samples from these subjects by enzyme-linked immunosorbent assay. HCV infections were found throughout the year, although most occurred during two periods, from June through September and from December through February. There were no marked seasonal differences in either the range of antibody rises obtained or in the HCV groups to which these antibody rises were directed. However, there were more HCV antibody rises during the summer than in the winter. The antibody duration varied considerably, but had a mean of 3.5 months. Finally, the frequency of HCV infection per person was calculated to be 1 per 7.8 months.

Human coronaviruses (HCVs) have been shown to cause mild upper respiratory tract infections in humans (2, 17, 21), with HCVs being responsible for up to 18% of colds (12, 17). At present, measurement of serum antibody rises is the most successful method for confirming HCV infections in humans, as other methods such as the detection or isolation of HCVs in nasal secretions are difficult and unreliable. Epidemiological studies measuring HCV infections by seroconversion with complement fixation and neutralization techniques have shown that these infections occur at all ages, are widespread throughout the world, and occur primarily during the winter and early spring (3, 4, 7–9, 20, 22).

Two antigenically distinct serological groups have been identified, which have been named after the prototype viruses, HCV 229E and HCV OC43, isolated by Hamre and Procknow (5) and McIntosh et al. (18), respectively. All HCVs so far isolated fall into one or other of these groups (16, 17, 19). Isolates within each HCV group are related to one another to various extents by complement fixation and neutralization tests (S. E. Reed, personal communication), but are all closely related by enzyme-linked immunosorbent assay (ELISA) (11, 16).

The incidence of infection with HCVs is complex, although a cyclic pattern has been discerned for both 229E and OC43 group viruses. HCV infections, as measured by antibody rises, were concentrated in certain years, with infections caused by 229E and OC43 group viruses generally occurring in 2- to 3-year cycles (3, 4, 7, 9, 10, 20, 22). Thus, in some years only sporadic HCV infections were observed, whereas in other years high infection rates were seen with either 229E or OC43 group viruses and only sporadic infections were seen with viruses belonging to the other HCV group. Complementary studies of antibody prevalence in the general population have shown that high antibody levels to 229E and OC43 viruses occur in a similar cyclic pattern (1).

The most sensitive reported method for detecting HCV antibody rises in human sera is the ELISA (11, 16). However, this method has only been used on paired sera from volunteers experimentally infected by intranasal inoculation of HCV isolates (11, 14, 16), although these studies have shown that rises in HCV antibody in human sera are related to mild upper respiratory tract infections or common colds. In this paper, the ELISA has been used for the first time to measure antibody rises to 229E and OC43 group viruses in sequential serum samples taken between 1976 and 1981 from adults living in or near London. The results obtained show the frequency, duration, and temporal distribution of HCV infections in this population.

MATERIALS AND METHODS

Adult sera. A total of 298 sequential serum samples were obtained between January 1976 and November

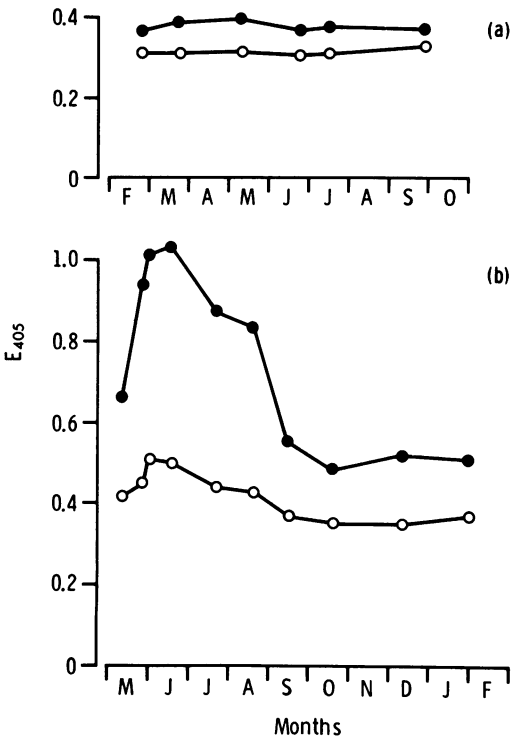

FIG. 1. HCV antibody, as represented by ELISA absorbance values, in sequential serum samples from two individuals shown in (a) and (b), respectively. Symbols: ● and ○, antibody detected to CV Paris and HCV 229E, respectively. Absorbance values were taken after 30 min at 405 nm. The months are shown from February (F) through October (O) in (a) and from May (M) through February (F) in (b).

1981 from 37 young adult volunteers and patients in the London area with a variety of non-respiratory infections. These sera were collected primarily for other trials, and full details of respiratory infections were not available. The subjects comprised 4 healthy volunteers, 29 patients with various rheumatic conditions, and 4 psychiatric patients. Wherever possible, the sequential serum samples used were taken from the individuals at intervals of less than 4 weeks, with the number of sequential serum samples per individual varying from 6 to 15. Although, the samples were collected over a long period, similar numbers of samples were collected for each calendar month (ranging from 23 to 27 per calendar month).

Viruses. Representative members of the two HCV antigenic groups were used. These were HCV 229E, the prototype virus of the 229E group (5), and CV Paris, of possible human origin isolated from the feces of a neonate with necrotizing enterocolitis (23) and a member of the OC43 group.

Preparation of antigens for the ELISA. HCV 229E was grown in MRC continuous cells as described previously (15). CV Paris was grown in HRT 18 cells in RPMI-1640 with 10% fetal calf serum (6). For both viruses, the cells were frozen and thawed once, and the resulting suspension was clarified at 2,000 × *g* for 30 min. Preparations of HCV 229E and CV Paris containing between 10^7 and 10^8 particles per ml, as determined by electron microscopy, were used. Clarified tissue culture fluids from MRC continuous cells and HRT 18 cells were used as control antigens.

ELISA procedure. The ELISA used for the detection of HCV antibody rises in human sera was described previously (11).

Suitable antigen and serum dilutions were selected by checkerboard titrations (11), and absorbance values were read after 30 min at 405 nm in a Flow Titertek Multiscan photometer. In all cases serum samples were tested against control antigens as well as HCV 229E and CV Paris; these control antigens consisted of clarified uninfected tissue culture fluids and Dulbecco phosphate-buffered saline A.

RESULTS

Detection of antibody rises in sequential sera by ELISA. Serum samples obtained from individuals over periods of up to 18 months were tested by ELISA for antibodies to HCV 229E and CV Paris; these viruses were representative members of the HCV 229E and HCV OC43 antigenic groups (6). Wherever possible, sequential samples taken at intervals of less than 4 weeks were used. Checkerboard titrations were done with a variety of serum and antigen dilutions to determine the optimum dilutions required to detect HCV antibodies (11). Serum and antigen dilutions of 1:50 were selected and used for all assays, and absorbance values were all read after 30 min at 405 mm.

All of the serum samples tested had HCV antibodies, although the antibody levels in the samples varied from individual to individual. The antibodies in these samples produced ELISA absorbance values ranging from 0.27 to 1.96, corresponding to titers of between 50 and 4,000 (6). In this study the relative antibody levels were measured by absorbance values with absorbances of twice the average control absorbances of 0.11 representing significant antibody (6). The level of antibody in the sequential sera of some individuals remained relatively constant over periods of up to 10 months. Figure 1a shows the antibody levels in serum samples from one individual. In this case the quantity of HCV 229E and CV Paris antibody remained relatively constant for over 7 months. On the other hand, antibody rises to one or other of the antigens were observed in serum samples taken from other individuals; Fig. 1b shows data from an individual whose sequential sera showed an antibody rise to CV Paris. A smaller rise to HCV 229E was also seen, due presumably to the detection of some antibodies common to HCV 229E and CV Paris (6). Antibody rises were expressed as ELISA ratios, which were the ratios of the maximum to the minimum absorbance values in the sequential serum samples at

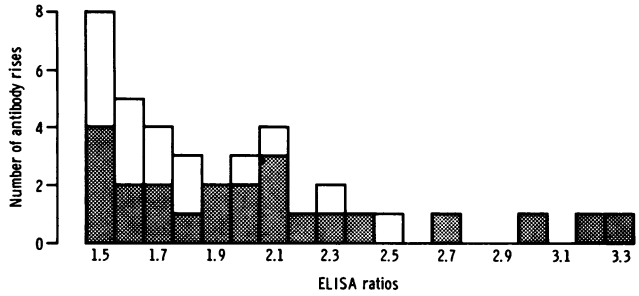

FIG. 2. Distribution of HCV antibody rises according to their ELISA ratios. Hatched and unhatched areas of the histogram represent antibody rises to CV Paris and HCV 229E, respectively.

the same serum and antigen dilutions. Thus, in Fig. 1b, antibody rises represented by ELISA ratios of 2.1 and 1.5 were obtained with CV Paris and HCV 229E, respectively.

Frequency and range of antibody rises. The ELISA detected 38 antibody rises in the sequential sera with ELISA ratios of 1.5 or more (Fig. 2). The total period that the sequential serum samples covered was 298 months. Thus, the frequency of HCV infections in these subjects between 1976 and 1981 as determined by HCV antibody rises with ELISA ratios of 1.5 or more was 1 per 7.8 months. Generally antibody rises were against either HCV 229E or CV Paris, although in four cases antibody rises of 1.5 or more were seen against both HCV 229E and CV Paris. In these cases the antibody rise was considered to be against the HCV with the higher ELISA ratio. Thus, 15 of the antibody rises were directed against HCV 229E, and 23 were directed against CV Paris. Figure 2 shows the range of ELISA ratios obtained for these antibody rises. A similar distribution of ratios was observed for both antigens, with ratios of up to 2.5 for HCV 229E and 3.3 for CV Paris.

Temporal distribution of HCV infections. Figure 3 shows the frequency of HCV antibody rises per month for a total of 298 months in sera taken between 1976 and 1981. HCV infections occurred throughout the year, although two main periods of antibody rises were seen associated with four summer months (June through September) and three winter months (December through February). If, for ease of analysis, the year is divided into two halves consisting of summer (May through October) and winter (November through April), then more antibody rises to both HCV groups were seen in the summer (22) than the winter (16). Furthermore, there were similar proportions of antibody rises to the two HCV groups in these periods, with nine (41%) and six (37%) antibody rises being to HCV 229E in the summer and winter, respectively. Finally, there were no differences in the antibody rises observed in different seasons; similar ELISA ratios for the two HCV groups were seen in the summer and winter (data not shown).

Antibody duration. The duration in months of raised HCV antibody in sera for six antibody rises was observed in five individuals. These antibody rises were selected because their sequential serum samples were taken at such times that adequately covered the period from the peak antibody levels to antibody levels similar to the basal levels seen before infection. The duration of HCV antibody in these sera varied from 2 to 5 months, with a mean of 3.5 months.

Repeated infection of individuals. Certain individuals appeared more prone than others to HCV infection. Unfortunately, it was difficult to reliably determine the proportion of such individuals in the population studied, as the number of subjects was too small. However, five individuals in the study developed two HCV infections within 12 months. Four of them had a second HCV infection with an HCV of the homologous group within 3 to 6 months (mean, 4 months) of the first infection, suggesting that

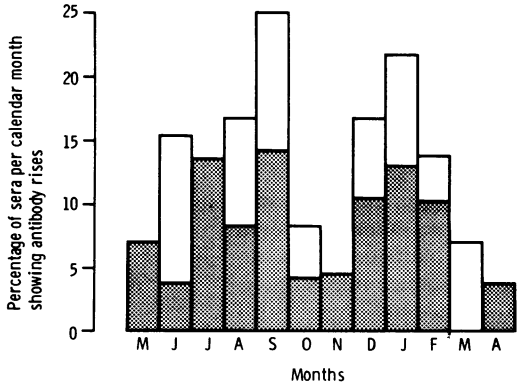

FIG. 3. Temporal distribution of HCV antibody rises. Hatched and unhatched areas of the histogram represent antibody rises to CV Paris and HCV 229E, respectively. The months are shown from May (M) through April (A).

HCV antibodies are only protective for about 4 months. In addition, one individual developed a 229E-type infection two months after an OC43-type infection, indicating that antibodies to one HCV group may not be protective against infection with viruses from the other HCV group.

DISCUSSION

This is the first report describing the detection by ELISA of HCV antibody rises in human sequential sera. Antibody rises to both HCV antigenic groups, 229E and OC43, were measured, and the antibody rises were expressed as ELISA ratios as described previously (11, 14, 16). In these previous studies, ELISA ratios of 2.0 or more were generally, but not always, associated with clinical symptoms (11, 16). However, antibody rises to HCVs with ELISA ratios of 1.5 or more were often seen in volunteers given HCVs but who did not develop respiratory disease. ELISA ratios from control antigens or serum samples from volunteers given saline instead of HCVs were always less than 1.5 (11, 14, 16).

Assuming that antibody rises with ELISA ratios of 1.5 or more represent genuine HCV infections, then 38 antibody rises were observed in the sequential sera. This represents a frequency of HCV infection of 1 per 7.8 months per subject. However, there were four cases with antibody rises, with ELISA ratios of 1.5 or greater, occurring to both HCV antigenic groups: these rises presumably reflect the detection of antibodies common to both HCV groups (6). In those cases, it was considered that the antibody rises were against the HCV with the highest ELISA ratio, although it is also possible that the highest antibody rise was not to the infecting virus or that these antibody rises are due to infections by viruses from the two HCV groups occurring at or near the same time. Thus, the figures for the frequency of HCV infections must only be taken as an indication of the possible frequency of infections, although they do show that HCV infections in humans are common.

Previous studies, all from the United States, have analyzed antibody rises to HCVs in paired sera from volunteers with respiratory infections or in sera taken at intervals of up to 6 months from volunteers with no clinical symptoms. These studies normally covered periods of several years and have shown a seasonal incidence of HCV infections due to both HCV 229E (3, 4, 9, 20) and HCV OC43 (10, 20, 22). Generally, HCV epidemics occurred during the winter or early spring, although in some studies the peak period of infection varied by several months (3, 4, 9, 10, 20, 22). However, other significant periods of HCV infection outside winter and early spring were also observed (3, 10, 20, 22), indicating that HCV infections can occur throughout the year.

The results in this paper show that HCV infections detected in individuals between 1976 and 1981 occurred throughout the year, although they were found more frequently during two periods between June and September and between December and February. The December through February period of infection corresponded to the winter period observed in the American studies. The pattern of HCV infection in the United Kingdom and the United States may indeed be different, but more likely reflect differences in the selection and analysis of samples. The ELISA used in this paper to detect antibody rises was much more sensitive, but less specific, than complement fixation and neutralization tests (11) used previously; this is reflected in the higher incidence of HCV infections observed in this study. It may be that HCV infections occur throughout the year, but only cause subclinical or very mild respiratory infections outside the winter and early spring. Certainly, antibody rises to HCVs have been frequently detected in paired sera from volunteers without clinical symptoms (7, 10). Finally, HCVs may cause infections that are not associated with respiratory diseases, such as gastroenteritis (13). This particularly applies to the HCV infections observed in the summer which may cause illnesses other than the respiratory illnesses previously associated with HCVs.

Several studies have shown that the majority of adults have serum antibodies to both 229E and OC43 group viruses. The proportion of adults whose sera contained these antibodies approached 100% when sensitive immunological tests such as radioimmunoassay (8) and ELISA (6) were used. Serum antibody levels appeared stable between infections (Fig. 1a). However, on infection they rapidly increased before gradually decreasing to basal levels (Fig. 1b) over 2 to 5 months, depending on the individual. We have previously shown that high antibody levels in a volunteer's sera lead to some immunity to HCV infection (11). The results in this paper confirm this suggestion, as subjects with an HCV infection appeared to be protected for at least 3 months against further infection with an HCV of the same type. However, one individual developed an OC43-type infection only 2 months after a 229E-type infection, suggesting that antibody to one HCV group may not be protective against infection with the other HCV group.

In conclusion, the results in this paper show that HCV infections are common and occur throughout the year. However, it was not possible to correlate these infections with clinical respiratory disease in humans. Future studies

are required to identify the range and severity of diseases caused by these viruses.

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